Use of aspartyl protease (VTPro) in meat-type broiler chickens fed diets with two levels of crude protein and essential amino acids. Performance data

1. Aim of the trial

The aim of this study was to compare the productive performance, after 42 days, of broiler chickens fed a standard control diet, two low-protein and amino acid diets (3% and 6% reduction, LP3 and LP6), and three levels of aspartyl protease (VTPro)[1] added to the LP6 diet. The diets are described in the Feeding Schedule chapter and their composition are shown in Tables 1 to 3.

2. Experimental design

2.1. Animal husbandry

The experiment was run in floor pens in Tekzol's Animal Experimentation Unit 1 located in Palmira (Valle del Cauca Department) - Colombia. This unit is placed in a thermally warm region, at 1.000 meters above sea level and near the Equator. Broiler density for this trial was set to 8 chickens per square meter. The birds were reared in a clean and disinfected place after a proper sanitary void previous to the start of the test. A layer of 10-15 cm of new wood shavings was used as litter; it was disinfected with quaternary ammonia and glutaraldehyde. The feeders were all of the same shape and size. Each feeder was identified with a colored marker indicating the kind of feed received. Chickens had feed and water available ad libitum throughout the trial.

With every diet change, the feeders were emptied, the remains, if any, weighed, and refilled with fresh feed. This process was carried out very carefully to avoid feed being spilled. General appearance of the animals, temperature, light, water, feed, litter condition and mortality were monitored and registered on a daily basis. Animals with very poor performance were removed from the trial, and the date of their removal and their weight were recorded for data adjustment.

2.2. Animals, housing and experimental design

One-day old, mixed sex Cobb 500 broiler chickens were obtained from a local supplier to be used for the trial. Animals were weighed, wing banded with a unique ID number and randomly allocated into pens according to treatment and replication. All animals were individually controlled during the experiment, effectively turning each animal into an experimental unit for weight gain.

The experiment structure followed a randomized complete design (RCD) and included 6 treatments. Those were: a positive control diet (PC); two negative control diets (LP3 and LP6) reformulated to have their essential amino acid content reduced by 3% and 6%, with concomitant reduction of crude protein levels; and three diets created from LP6 diet. These contained 50, 100, and 150 ppm aspartyl protease (VTPRO). Each treatment had 5 replicates of 24 animals each. Feeders were color-coded according to the feed they should contain and treatments and replicates were distributed into pens as follows:

Code	Control	LP3	LP6	50 ppm	100 ppm	150 ppm		
Letter	A	B	C	D	E	F		
Color	White	Blue	Green	Red	Orange	Yellow		
Pen	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Diet/Replica	**B4**	**A2**	**D3**	**A5**	**A4**	**E3**	**F4**	**E1**
Pen	**16**	**15**	**14**	**13**	**12**	**11**	**10**	**9**
Diet/Replica	**F3**	**E4**	**B5**	**D4**	**B1**	**F1**	**C5**	**D5**
Pen	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Diet/Replica		**F2**	**C3**	**A3**	**B2**	**C4**	**E5**	**B3**
Pen	**32**	**31**	**30**	**29**	**28**	**27**	**26**	**25**

[1] VTPro. Aspartyl protease manufactured by Guangdong VTR Bio-Tech Co. Ltd., Guangdong, China

Diet/Replica	A1	D1	E2	C2	D2	C1	F5

2.3. Feeding program

The feeding program had three phases: pre-starter phase, from day 1 to 10; starter phase, from day 11 to 23; and a growing phase from day 24 to 42. Feed was supplied ad libitum. The control diet was a ration based on corn and soybean meal, meeting the nutritive requirements of the strain (Cobb 500) by its producer for as hatched birds (Cobb-Vantress, 2018). Tables 1 to 3 show the feed formulation and nutritional composition of all diets. Diets contained 1% Celite©, a source of indigestible ash for nutrient digestibility studies.

Table 1. Ingredient and nutritive composition of the experimental pre-starter diets.

Ingredient	PC	LP3	LP6	Nutrient	PC	LP3	LP6
Yellow corn	561.74	585.64	608.55	Crude protein, %	24.26	22.80	21.44
SBM	259.24	259.19	259.11	Ether extract, %	4.41	4.60	4.80
Corn gluten meal	55.56	33.05	10.00	Calcium, %	0.90	0.90	0.90
Blood meal	42.58	40.00	39.24	Available P, %	0.45	0.45	0.45
Palm oil	21.53	23.16	24.89	Crude fiber, %	2.05	2.08	2.10
DCP	24.32	24.35	24.37	ME, kcal/kg	3000	3000	3000
Celite	10.00	10.00	10.00	Linoleic acid, %	1.48	1.52	1.55
Limestone	6.07	6.06	6.05	Electrolytes, mEq/kg	200	200	200
Vit-Min premix	6.00	6.00	6.00	Sodium, %	0.229	0.219	0.214
Bicarbonate Na	2.80	2.76	2.58	Starch, %	35.75	36.92	38.02
DL methionine	3.77	3.70	3.50	Chloride, %	0.285	0.272	0.266
Choline HCl 60%	1.20	1.20	1.20	Potassium, %	0.780	0.780	0.780
L-lysine HCl	1.55	1.52	1.26	Lysine, %	1.260	1.222	1.180
L-threonine	0.84	0.77	0.65	Methionine, %	0.707	0.672	0.625
Salt	2.79	2.60	2.60	Met+cys, %	1.008	0.953	0.887
Total	1000.0	1000.0	1000.0	Threonine, %	0.856	0.806	0.755
				Tryptophan, %	0.243	0.234	0.227
				Arginine, %	1.226	1.184	1.146
				Isoleucine, %	0.781	0.733	0.684
				Leucine, %	2.227	1.991	1.764
				Valine	1.148	1.075	1.014

Table 2. Ingredient and nutritive composition of the experimental starter diets.

Ingredient	PC	LP3	LP6	Nutrient	PC	LP3	LP6
Yellow corn	567.65	561.63	587.47	Crude protein, %	22.32	21.51	20.58
SBM	258.74	281.30	262.56	Ether extract, %	6.481	6.64	6.77
Corn gluten meal	45.00	35.58	29.53	Calcium, %	0.78	0.78	0.78
Blood meal	20.00	10.00	10.00	Available P, %	0.42	0.42	0.42
Palm oil	57.04	61.98	59.50	Crude fiber, %	2.06	2.08	2.10
DCP	19.85	19.65	19.86	ME, kcal/kg	3100	3100	3100
Celite	10.00	10.00	10.00	Linoleic acid, %	1.70	1.72	1.75
Limestone	5.22	5.19	5.19	Electrolytes, mEq/kg	200	200	200
Vit-Min premix	6.00	6.00	6.00	Sodium, %	0.214	0.201	0.213
Bicarbonate Na	2.00	1.32	2.00	Starch, %	36.11	36.59	37.25
DL methionine	3.19	2.91	2.72	Chloride, %	0.260	0.243	0.260
Choline HCl 60%	0.85	0.85	0.85	Potassium, %	0.783	0.790	0.790
L-lysine HCl	1.11	1.05	1.29	Lysine, %	1.275	1.239	1.190
L-threonine	0.47	0.18	0.12	Methionine, %	1.120	1.090	1.050
Salt	2.88	2.37	2.93	Met+cys, %	0.649	0.610	0.577
Total	1000.0	1000.0	1000.0	Threonine, %	0.932	0.885	0.844
				Tryptophan, %	0.772	0.730	0.682
				Arginine, %	0.226	0.220	0.211
				Isoleucine, %	1.176	1.158	1.132
				Leucine, %	0.750	0.737	0.719
				Valine	1.979	1.863	1.743

Table 3. Ingredient and nutritive composition of the experimental grower diets.

Ingredient	PC	LP3	LP6	Nutrient	PC	LP3	LP6
Yellow corn	570.77	580.38	592.80	Crude protein, %	21.46	20.95	19.89
SBM	263.19	266.91	266.97	Ether extract, %	7.93	8.41	8.21
Corn gluten meal	40.00	31.46	23.35	Calcium, %	0.76	0.76	0.76
Blood meal	30.00	24.43	18.89	Available P, %	0.38	0.38	0.38
Palm oil	42.30	43.78	44.90	Crude fibre, %	2.04	2.09	2.08
DCP	22.39	22.37	22.40	ME, kcal/kg	3180	3180	3180
Celite	10.00	10.00	10.00	Linoleic acid, %	1.85	1.89	1.89
Limestone	4.09	4.08	4.06	Electrolites, mEq/kg	190	190	190
Vit-Min premix	6.00	6.00	6.00	Sodium, %	0.201	0.160	0.199
Bicarbonate Na	2.50	2.47	2.50	Starch, %	35.97	35.52	36.99
DL methionine	3.45	3.18	2.98	Chloride, %	0.260	0.223	0.260
Choline HCl 60%	1.00	1.00	1.00	Potassium, %	0.770	0.812	0.777
L-lysine HCl	0.97	1.07	1.13	Lisyne, %	1.050	1.020	0.990
L-threonine	0.57	0.38	0.18	Methionine, %	0.619	0.583	0.549
Salt	2.76	2.50	2.83	Met+cys, %	0.899	0.861	0.814
Total	1000.0	1000.0	1000.0	Threonine, %	0.735	0.693	0.653
				Tryptophan, %	0.213	0.211	0.200
				Arginine, %	1.141	1.163	1.101
				Isoleucine, %	0.748	0.765	0.720
				Leucine, %	1.929	1.815	1.712
				Valine	0.953	0.897	0.851

2.4. Records

Live weight. All animals were pool weighed according to the protocol followed in previous tests, at day 0 (initial), at diet changes at 11 and 22 days. Final weighing was at day 42, end of the experiment. All weights were taken using an electronic scale with ±1 g accuracy.

Apparent consumption. Feed disappearance was recorded at diet changes. Average daily intake, gain and period feed conversion ratio were calculated per pen.

The conversion ratio adjusted by mortality was calculated as follows: total feed intake per period and pen/(total live weight of the pen + weight of the dead birds per pen)–total live weight of the pen in a former period.

Mortality was recorded and dead animals weighed to adjust the period performance taking into account this parameter.

Records were kept during the experiment on the number of animals with thick stools or any other contingency.

3. Statistical analysis

The statistical program SPSS was used to carry out an analysis of variance (ANOVA) on the data obtained, to assess the effect of the experimental diets on chicken performance. Under normal circumstances, the general lineal model with multivariate analysis of variance was used. All declarations of significance were based on a probability level of $p < 0.05$. Assuming equal variances (Levene not significant) Duncan Multiple Range (DMR) test or Tukey (HSD) were used to separate the means. If Levene was significant for any measurements, Dunnett T3 or Kruskal-Wallis tests would be applied. Be aware that the diet to compare to the treatments is LP3.

5. Results

The experiment developed uneventfully. There were no problems and animals grew at a good pace.

Body weight

Tables 4 to 6 show the mean body weight of all treatments, for male and female birds, and for the overall experimental population. The statistical analysis compares LP3 against LP6+VTPro treatments.

All animals, irrespective of sex, experience a linear weight decrease with the decrease of essential amino acids and crude protein in the diet.

Table 4. Weekly body weight of male chickens in the experiment

Wk	PC	LP3	LP6	VTP50	VTP100	VTP150
1	180	175^{a}	165^{b}	172^{a}	173^{a}	168^{b}
2	457	424^{a}	404^{b}	419^{a}	431^{a}	414^{a}
3	956	900^{a}	860^{b}	812^{a}	908^{a}	895^{a}
4	1601	1525^{a}	1463^{b}	1535^{a}	1513^{a}	1505^{a}
5	2269	2184^{a}	2056^{b}	2074^{b}	2196^{a}	2130^{a}
6	2914	2788^{a}	2630^{b}	2628^{b}	2787^{a}	2746^{a}

Table 5. Weekly body weight of female chickens in the experiment

Wk	PC	LP3	LP6	VTP50	VTP100	VTP150
1	171	168^{a}	156^{b}	165^{a}	172^{a}	161^{a}
2	429	408^{a}	379^{b}	399^{a}	414^{a}	384^{a}
3	865	852^{a}	778^{b}	820^{a}	835^{a}	800^{b}
4	1400	1403^{a}	1285^{b}	1340^{b}	1359^{a}	1313^{b}
5	1905	1889^{a}	1770^{b}	1791^{b}	1901^{a}	1846^{b}
6	2510	2440^{a}	2211^{b}	2294^{b}	2416^{a}	2321^{b}

Table 6. Overall weekly body weight in the experiment

Week	PC	LP3	LP6	VTP50	VTP100	VTP150
1	174	172^{a}	162^{b}	168^{a}	174^{a}	165^{b}
2	443	416^{a}	391^{b}	409^{a}	423^{a}	399^{b}
3	911	876^{a}	818^{b}	866^{a}	874^{a}	848^{b}
4	1508	1462^{a}	1371^{b}	1435^{a}	1437^{a}	1410^{b}
5	2086	2033^{a}	1905^{b}	1930^{b}	2047^{a}	1982^{a}
6	2706	2611^{a}	2406^{b}	2456^{b}	2596^{a}	2537^{a}

a, b: means with different superscripts are significantly different ($p<0.05$)

Throughout the experiment, the LP6 diet achieved significantly ($p<0.05$) lower weekly weight than most other diets.

When compared against LP3, the body weight of birds fed the VTP100 diet was not different ($p<0.05$). In body weight response, we can say that the VTP100 diet (having 6% lower values of essential amino acids and crude protein, supplemented with 150 ppm aspartyl protease) was identical to LP3 diet.

The body weight of birds fed diet VTP150 was not different ($p<0.05$) than both LP3 and VTP100. There is literature evidence that excess protease can be detrimental for the bird growth, and the results of this experiment seems to suggest that. Only in the two last weeks of the experiment, the body weight of birds fed this diet was not different ($p<0.05$) to LP3, but the calculated p-values for these two weeks were just 0.167 and 0.135 for weeks 5 and 6, respectively.

Diet VTP50 is clearly not sufficient to compensate the aggressive reduction of amino acids and crude protein executed in this experiment, the body weight values being significantly ($p<0.05$) lower than either LP3 or VTP100.

Feed intake

Feed intake for all treatments appear in Tables 7 to 9. There were no significant differences ($p<0.05$) in feed intake among treatments in the cumulative values to day 42.

Table 7. Weekly feed intake of male chickens in the experiment

Wk	PC	LP3	LP6	VTP50	VTP100	VTP150
1	158	151	157	148	152	149
2	531	514	527	507	520	503
3	1191	1171	1178	1154	1172	1139
4	2267	2229	2202	2189	2212	2139
5	3381	3313	3376	3281	3340	3206
6	4815	4601	4665	4548	4620	4453

Table 8. Weekly feed intake of female chickens in the experiment

Wk	PC	LP3	LP6	VTP50	VTP100	VTP150
1	147	148	151	140	148	144
2	506	507	515	486	509	488
3	1112	1120	1126	1075	1113	1092
4	2012	1998	2007	1921	2003	1972
5	3148	2918	2941	2871	2949	2937
6	4362	4138	4074	4011	4062	4081

Table 9. Overall feed intake in the experiment

Wk	PC	LP3	LP6	VTP50	VTP100	VTP150
1	153	150	154	144	150	147
2	518	511	521	497	514	496
3	1152	1146	1152	1114	1142	1116
4	2140	2114	2105	2055	2108	2056
5	3265	3115	3159	3076	3144	3072
6	4588	4369	4369	4280	4341	4267

FCR

Tables 10 to 12show the FCR values for the trial.

Table 10. Weekly FCR of male chickens in the experiment

Wk	PC	LP3	LP6	VTP50	VTP100	VTP150
1	0.872	0.866	0.950	0.860	0.879	0.887
2	1.161	1.212	1.305	1.212	1.206	1.214
3	1.246	1.302	1.369	1.421	1.290	1.272
4	1.417	1.462	1.506	1.425	1.461	1.421
5	1.489	1.518	1.644	1.581	1.521	1.504
6	1.653	1.650	1.774	1.731	1.658	1.622

Table 11. Weekly FCR of female chickens in the experiment

Wk	PC	LP3	LP6	VTP50	VTP100	VTP150
1	0.862	0.879	0.967	0.845	0.858	0.899
2	1.179	1.243	1.358	1.220	1.230	1.269
3	1.286	1.316	1.448	1.311	1.333	1.365
4	1.436	1.424	1.562	1.433	1.473	1.502
5	1.651	1.544	1.661	1.602	1.551	1.591

6	1.735	1.695	1.845	1.748	1.682	1.758

Table 12. Overall FCR in the experiment

Wk	PC	LP3	LP6	VTP50	VTP100	VTP150
1	0.867	0.873	0.959	0.853	0.868	0.893
2	1.170	1.228	1.332	1.216	1.218	1.242
3	1.266	1.309	1.408	1.366	1.312	1.319
4	1.427	1.443	1.534	1.429	1.467	1.461
5	1.570	1.531	1.652	1.592	1.536	1.547
6	1.694	1.672	1.809	1.739	1.670	1.690

There were no significant differences in FCR among groups.

Conclusion

Decreasing protein and amino acid content in the diet causes a linear decrease in body weight of meat-type broiler chickens. The experiment tried recovering chicken performance to the level of LP3 diet or better, by adding graded doses of aspartyl protease (VTPro) to the LP6 diet.

Chickens fed the LP6 diets containing increasing doses of aspartyl protease did show a quadratic ($p<0.05$) response for body weight with the optimum value at 100 ppm in males, females, or the overall population of the supplemented diets.

Diet VTP100 (Lp6 + 100 ppm of VTPro) totally reverses the negative growth impact of a 3% reduction in essential amino acids and crude protein in the diet, and matches performance with diet LP3.

There were no significant ($p<0.05$) differences in feed intake across groups, nor there were significant ($p<0.05$) differences in FCR. Numerically, at the end of the experiment the best FCR value was for diet VTP100.

We can conclude that decreasing 3% essential amino acids and crude protein in the diet of broiler chickens up to 42 days of age will reduce body weight at the end of the growing phase. Adding 100 ppm of an aspartyl protease (VTPro) will completely recover weight gain with no other performance implications. This may potentially improve the economy of broiler production.

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